A rapid modified gas chromatographic method for carbohydrate analysis of wood pulps

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ABSTRACT

The current procedure of TAPPI Test Method T 249 cm-85 for carbohydrate analysis requires acetylation of alditols. Preparation of alditol acetates involves a series of evaporation steps. This investigation is an attempt to improve the efficiency of this gas chromatography method. Using l-methylimidazole as a catalyst, acetylation of alditols in the presence of borates has been successfully accomplished. This eliminates the need for the evaporation steps. Analysis of a number of different pulp samples, using the modified method, showed accurate and repeatable results. The monosaccharide compositions were comparable with the conventional TAPPI method.

Application:

A modified method for carbohydrate analysis makes it possible to characterize wood pulp carbohydrates faster than with TAPPI Test Method T249 cm-85, without losing accuracy.

GAS CHROMATOGRAPHY OF alditol acetates has been used for carbohydrate analysis of wood pulps since 1970 *(1)*. The method is standardized as TAPPI Test Method T 249 cm-85 *(2)*. Several efforts *(3–8)* have been made to improve the efficiency of carbohydrate analysis. The efforts mainly include the utilization of anion-exchange high-performance liquid chromatography (HPLC). Analysis by HPLC is rapid due to the simplicity of the sample preparation. Judged from chromatograms, however, the incomplete separation of minor components in wood pulps, including arabinose, xylose, and galactose, may undermine the accuracy of a quantitative analysis.

The complexity of TAPPI T 249 stems from the long and tedious evaporation step in preparing alditol acetates. After hydrolysis of wood or wood pulps, alditols are normally prepared in an aqueous solution by reduction with sodium borohydride. Borates are formed when the excess of sodium borohydride is decomposed with acetic acid *(9)*. In the presence of borates, acid-catalyzed acetylation leads to the formation of a number of artifacts. These artifacts have retention times similar to alditol acetates in a gas chromatography column *(10)*. Therefore, the removal of borate as its volatile trimethyl ester is essential before acetylation. To assist in the formation of trimethyl borate, methanol must be repeatedly added to the residue obtained on concentrating the aqueous solution to dryness *(11)*. Therefore, there is a lengthy and tedious process of repeated evaporation in the current TAPPI Test Method T 249 cm-85.

The compound 1-methylimidazole was first used as a catalyst for acetylation of hydroxy compounds by Connors and Pandit in 1978 *(12)*. They found that the catalytic activity of 1-methylimidazole was about 4 x 10^2 times greater than pyridine. In 1983, Blakeney *et al. (13)* reported the successful use of 1-methylimidazole as a catalyst for quantitative acetylation of alditols in the presence of borate. The method was applied to the microanalysis of monosaccharides in endosperm cells of Italian ryegrass (*Lolium multiflorum* Lam.).

In the work reported here, we studied the use of 1-methylimidazole as a catalyst for acetylation of alditols during analysis of wood pulps. Our primary objective is to develop an effective and accurate, but less complex, method for carbohydrate analysis of wood pulps that is comparable with the TAPPI Test Method.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5890 gas chromatograph, equipped with a 7673A automatic sampler and an HP 3396A integrator, was used to quantitatively analyze neutral carbohydrate components in wood pulps. Alditol acetates were separated on a DB-225 fused silica capillary column (30 m x 0.318 mm). The column has a stationary phase of (50% cyanopropylphenyl)-methylpolysiloxane. Its film thickness is 0.15 µm. Oven temperatures were programmed. The initial temperature was kept at 60°C for 0.5 min and then raised at 15°/min to 220°C, where it was kept for 13 min. The injection and detection port temperatures were 225°C and 250°C, respectively.

Reagents

ß-D(+) glucose, D(+) mannose, D(+) galactose, D(+) xylose, and myo-inositol were purchased from Sigma Chemical, and L-arabinose was obtained from Aldrich Chemical. These monosaccharides and myo-inositol were dried in a vacuum desiccator over pentaphosphor oxide for at least 72 hours. The inositol solution was prepared by dissolving 2.5 g of dried myo-inositol in 250 mL

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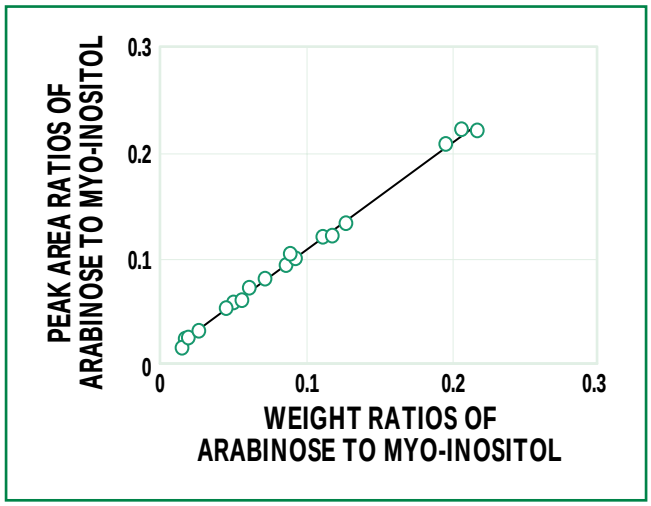
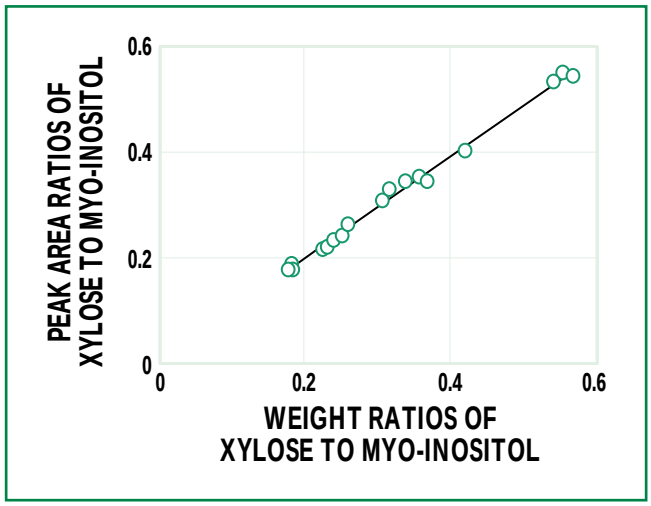
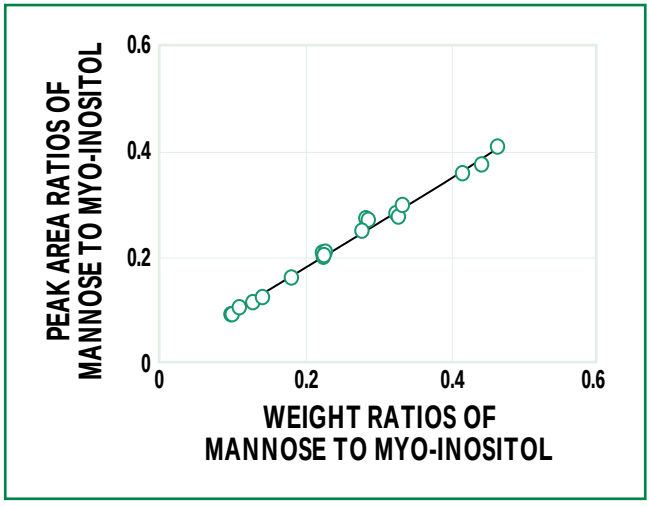


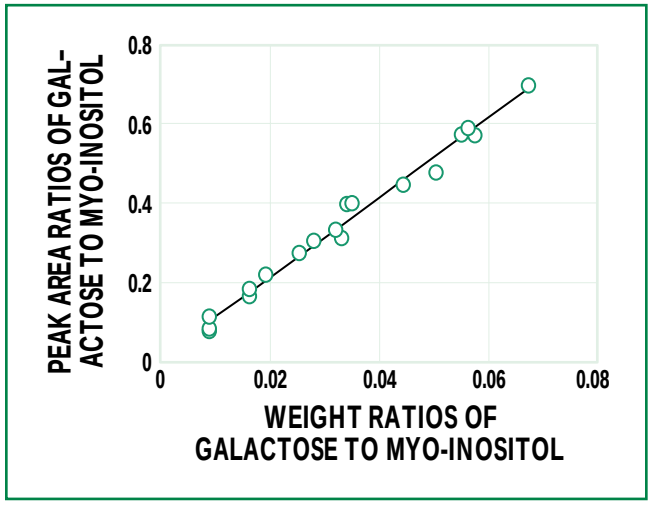
1. Calibration line for arabinose with myo-inositol as internal standard



2. Calibration line for xylose with myo-inositol as internal standard



3. Calibration line for mannose with myo-inositol as internal standard



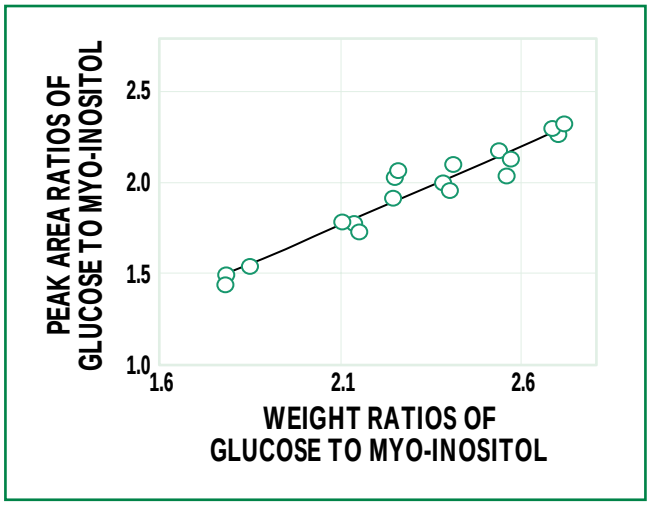
4. Calibration line for galactose with myo-inositol as internal standard

of distilled water. HPLC-grade dichloromethane and AR-grade acetic acid were obtained from Mallinckrodt Chemical. ACS-grade acetic anhydride was obtained from Fisher Scientific. GR-grade ammonium hydroxide was purchased from EM Science. To prepare 72% sulfuric acid, 665 mL of concentrated sulfuric acid (95.9%, sp. gr. 1.84) were slowly poured into 300 mL of distilled water in a 1000-mL volumetric flask. After cooling, up to 1000 mL were made. The strength was adjusted by measuring the specific gravity to 1.6338.

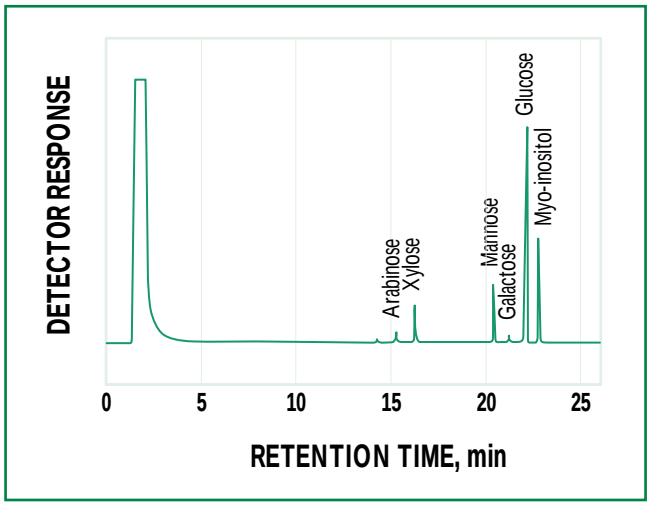
Procedure

Obtain a sample of pulp weighing approximately 5 g, and grind the sample to pass a 40-mesh screen. Allow the sample to reach moisture equilibrium. Weigh two test specimens of 0.35±0.01 g to the nearest of 0.1 mg into 150-mL beakers. Add exactly 3 mL of 72% sulfuric acid to the specimen with a pipet. Stir the contents of the beaker with a glass rod until the specimen starts to dissolve. Place the beaker in a 30±0.5°C water bath for 1 hour. Stir the specimen every 5–10 min. At the end of the hour, add 84 mL of distilled water. Cover the beaker with a watch glass, and heat it in an autoclave at 121°C (15 psi) for 1 hour.

Add 10 mL of myo-inositol internal standard solution to the beaker. After mixing, cool the specimen solution. Filter off the lignin residue through a 40-mL sintered glass filter crucible (medium). Apply a slight vacuum. The final volume of filtrate is adjusted to 140 mL. Add approximately 11.0 mL ammonium hydroxide solution (28.0–30.0% ammonia) to the filtrate to make 1*M* with respect to ammonia. Pipet a 2-mL aliquot into a 125-mL Erlenmeyer flask. Weigh 35±1 mg sodium borohydride, and add it to the flask. Place



5. Calibration line for glucose with myo-inositol as internal standard



6. A typical chromatogram of a pulp sample

the flask in a 40±1°C water bath for 90 min. Stir the contents occasionally. After reduction, decompose the excess of sodium borohydride by adding 1 mL glacial acetic acid one drop at a time. Add 2 mL 1-methylimidazole and a stirring bar, immediately followed by 20 mL acetic anhydride, to the reduced monosaccharides. Agitate the mixture continuously with a magnetic stirrer at room temperature for 20 min. The heat generated will promote the acetylation to completion.

After 20 min, add 30 g crushed ice and 70 g distilled water to the flask; continue to stir for at least 20 min. Transfer the mixture to a 250-mL separatory funnel and extract successively with 10-, 5-, and 5-mL portions of dichloromethane, and combine the dichloromethane extracts in a 250-mL beaker. Put the beaker in a well-ventilated fume hood for at least 1 hour. Add 2 mL of dichloromethane to the dried alditol acetate mixture. Store the solution in a 4-mL glass vial at –20°C. Prior to running the gas chromatography, dilute 0.2 mL alditol acetate solution into 1 mL dichloromethane. Inject 1 µL of diluted alditol acetate solution routinely, and determine the peak area for each of the five monosaccharides and the internal standard. The percent of each component as polysaccharide is calculated according to the equation given in TAPPI T 249 cm-85.

Monosaccharide	Wt. range, mg	*k*-factor	Corr. coefficient
Arabinose	1.4–21.8	1.013	0.999
Xylose	17.8–56.5	0.982	0.998
Mannose	9.2–46.4	0.859	0.997
Galactose	0.9–6.7	1.027	0.994
Glucose	178.3–276.1	0.867	0.965

I. Calibration factors for five monosaccharides

Calibration

An internal standard, myo-inositol, was added to a mixture of five monosaccharides to establish detector response factors for each component and to correct for minor decomposition during hydrolysis. The mixture was then subjected to a treatment identical to that of the pulp samples. Eighteen standard mixtures covering a wide range of composition in wood pulps were run to obtain calibration factors. Chromatographic peak area ratios (monosaccharide to myo-inositol) were plotted against weight ratios (monosaccharide to myo-inositol) (**Figs. 1–5**). Calibration factors (*k*-factors) were obtained by calculating the slope of regression lines. The correlation coefficients indicate that the calibration lines are highly linear within the range of the monosaccharide weight tested. The applied amount of each monosaccharide and corresponding calibration factors are listed in **Table I**.

RESULTS AND DISCUSSION

A hemlock (*Tsuga heterophylla* [Raf.] Sarg.) thermomechanical pulp was delignified and treated with caustic to different degrees to obtain pulps with wide-ranging lignin and carbohydrate contents. These pulps were then analyzed to determine their carbohydrate compositions. The results are listed in **Table II**. A typical chromatogram is shown in **Fig. 6**.

The successful preparation of alditol acetates is indicated by a smooth baseline and well separated monosaccharide peaks in the chromatogram. The complete separation, coupled with the elimination of peak tailing, allows accurate analysis of monosaccharides derived from wood pulps, including minor components. The excellent separation of alditol acetates can also be attributed to the use of capillary column DB-225.

Pulp	Test specimen	Arabinan, %	Xylan, %	Mannan, %	Galactan, %	Glucan, %
Sample A	1st	1.25	6.31	14.56	1.43	60.33
	2nd	1.12	5.95	14.07	1.26	59.94
Sample B	1st	0.66	3.40	15.17	1.00	70.50
	2nd	0.67	3.52	15.51	1.00	72.01
Sample C	1st	0.34	0.79	9.56	0.81	79.94
	2nd	0.32	0.81	9.74	0.82	82.45
Sample D	1st	1.16	5.67	13.91	1.50	52.97
	2nd	1.13	5.71	14.04	1.51	53.34
Sample E	1st	0.88	4.19	16.14	1.41	62.00
	2nd	0.88	4.18	16.14	1.39	63.81
Sample F	1st	0.66	2.26	14.55	1.31	70.50
	2nd	0.63	2.30	15.00	1.38	72.67
Sample G	1st	1.03	5.05	13.39	1.58	48.44
	2nd	1.00	4.96	12.96	1.46	47.05
Sample H	1st	1.01	5.10	14.48	1.49	52.36
	2nd	1.01	5.07	14.47	1.50	53.03
Sample I	1st	0.86	3.69	14.33	1.45	55.72
	2nd	0.93	3.74	14.56	1.44	56.60
Sample J	1st	1.22	4.86	11.95	1.64	42.36
	2nd	1.24	4.95	12.27	1.70	43.75
Avg. rel. std. dev., %[a]	---	2.9	1.3	1.3	2.1	1.2
Avg. repeatability, %[b]	---	7.9	3.7	3.6	5.8	3.4

[a]Obtained by dividing the standard deviations by the test averages and multiplying by 100.
[b]Obtained by multiplying the relative standard deviation by 2.77.

II. Analysis of ten different pulp samples using the modified gas chromatographic method

To establish the precision of the modified method, duplicate analyses were made of each of the ten different pulp samples with the same operator and apparatus, and the two test results were obtained in immediate succession on the same day. The percent repeatability was then calculated. According to the definition given by the TAPPI precision statement for test methods *(14),* "repeatability is a limit within which agreement may be expected 95% of the time between two test results obtained under essentially the same conditions and from the same homogeneous sample of material." Mathematically, the repeatability is 2.77 times the corresponding standard deviation. The percent repeatability equals the repeatability multiplied by 100 and divided by the test average for that monosaccharide. The average percent repeatability of the ten samples is listed in Table II.

KEYWORDS
Acetylation, alditols, analysis, carbohydrates, catalysts, gas chromatography, monosaccharides, pulps, test methods.

The low percent standard deviation and repeatability data indicate that the modified method is precise and repeatable. Also, the statistical analysis of the modified method with low variations compares favorably with Borchardt and Piper's analysis *(1)* using the TAPPI Test Method.

To check the validity of the modified method, results for three pulp samples were compared with TAPPI T 249. The analysis by the TAPPI Test Method was conducted in the laboratory of Integrated Paper Services, Appleton, WI. In **Table III**, the test results from TAPPI T 249 are compared with the corresponding results from the modified method. The percent compositions of the different monosaccharides are fairly close to those obtained by the TAPPI method. A paired t-test, based on the differ-

ences between test results by the two different methods, gives smaller t-values than the critical value at 95% confidence level for all five monosaccharide components. This suggests that the modified and conventional methods give essentially the same results on the same sample; at least there is no evidence to show that they are different.

There are several advantages of using this modified method. After acid hydrolysis, ammonium hydroxide is used to neutralize the hydrolyzate instead of barium hydroxide. Subsequently, a centrifuge is not needed to remove barium sulfate precipitate. With 1-methylimidazole as a catalyst, acetylation can be finished in 10–20 min at room temperature, compared with 1 hour at 60°C with sulfuric acid as catalyst. The use of a rotary evaporator is avoided, because a high-temperature evaporation step is not required. The entire operation is performed in glassware. Once calibration factors are established, an analysis of eight samples can be completed in an 8-hour day.

CONCLUSIONS

From this investigation, it appears that 1-methylimidazole can act as a reliable catalyst for acetylation of alditols during carbohydrate analysis of wood pulps. The use of the catalyst greatly simplifies the preparation of alditol acetates, and acetylation is fast and complete. Also, statistical analysis of ten different pulp samples showed that the modified method is accurate, repeatable, and comparable with the conventional TAPPI Test Method T 249 cm-85. Further analysis on many other types of pulp is recommended to verify wider applicability of this modified method and to achieve universal acceptance. **TJ**

Parameter	Testing method	Arabinan, %	Xylan, %	Mannan, %	Galactan, %	Glucan %
Sample A	Modified	1.23	6.13	14.32	1.35	60.14
	T 249	0.9	7.7	13.3	1.2	67.0
Sample B	Modified	0.67	3.46	15.34	1.00	71.26
	T 249	0.9	3.9	15.6	1.1	71.1
Sample F	Modified	0.65	2.28	14.78	1.35	71.59
	T 249	0.7	2.1	14.9	1.4	70.8
t-value*	---	0.101	–1.191	0.526	0	–0.804

*Critical t-value at 95% confidence level = 4.30.

III. Comparison of results obtained by TAPPI T 249 and the modified method

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The authors wish to thank Thomas Krick for his assistance with the operation of the gas chromatograph, Birgit Grund for her comments on the statistical analysis of the data, and Doug Dugal for his review of the manuscript. Thanks are also due to the Minnesota Agricultural Experiment Station for funding this project.

Received for review Feb. 4, 1997.
Accepted March 8, 1997.

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